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# Toxicology and Carcinogenesis Studies of Androstenedione

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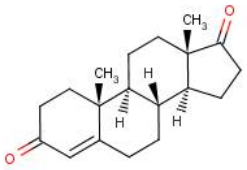
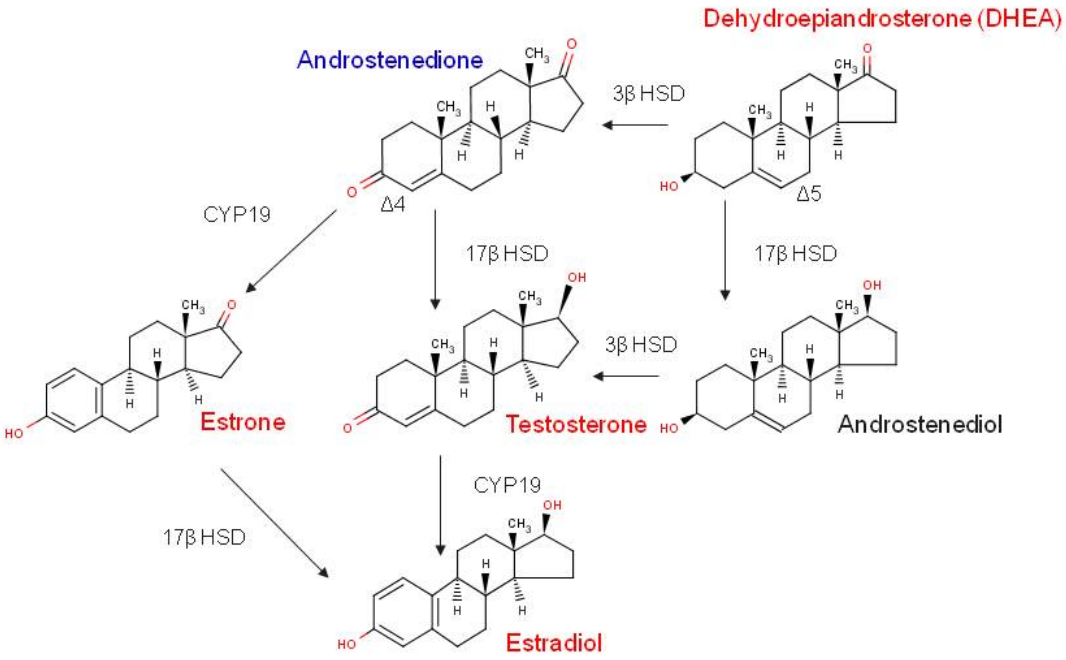
# Nomination and Exposure




- Androstenedione (Andro) was nominated for study by NCI due to health concerns from its chronic use by athletes and bodybuilders
- Androstenedione was promoted as an anabolic hormone (of questionable efficacy) in dietary supplements. Common routes were oral, sublingual, and dermal
- Recommended doses ranged from 100 - 1200 mg/day (70 kg person = 1.4 – 17.1 mg/kg/day)
- In 2004, androstenedione was banned for sale in dietary supplements due to Anabolic Steroid Control Act

# Metabolism


Dehydroepiandrosterone (DHEA)
Androstenedione
3β HSD
Δ4
Δ5
CYP19
17β HSD
17β HSD
3β HSD
Estrone
Testosterone
Androstenediol
CYP19
17β HSD
Estradiol

# Study Rationale and Objectives

- Objective: characterize the chronic toxicity and carcinogenic activity of androstenedione in F344/N rats and B6C3F1 mice

- Study design:
  - Genetic toxicity studies (in vitro and in vivo)
  - Subchronic gavage studies in F344/N rats and B6C3F1 mice
  - Chronic gavage studies in F344/N rats and B6C3F1 mice

# Subchronic and Genetic Toxicity Studies

- **Doses: 0, 1, 5, 10, 20, and 50 mg/kg/d (aqueous 0.5% methyl cellulose)**

- **2 week studies in rats and mice:**
  - No dose limiting effects
  - Liver peroxisome and cell proliferation assays were negative in mice and rats

- **3 month studies in rats and mice:**
  - No dose limiting effects; treatment related effects in adrenal X-zone of female mice
  - Reduction in spermatozoa numbers (rat) and motility (mouse) suggest possible adverse effects on reproduction

- **Genetic Toxicity:**
  - Androstenedione was negative in NTP bacterial assays
  - Bone marrow micronucleus assay was negative in rats
  - Peripheral blood erythrocyte micronucleus test was negative in male mice, equivocal in females (50 mg/kg)

# Androstenedione Doses for Chronic Exposure Study

- Dose levels selected for chronic exposure (2 year) were:
  - 0,10, 20 and 50 mg/kg/d for rats and male mice due to:
    - No observed intolerable dose effects in subchronic studies
    - Limit of gavageability at 50 mg/kg/d
  - 0, 2, 10, and 50 mg/kg/d for female mice:
    - suspected ovarian atrophy in the 3 month study
    - not confirmed by PWG

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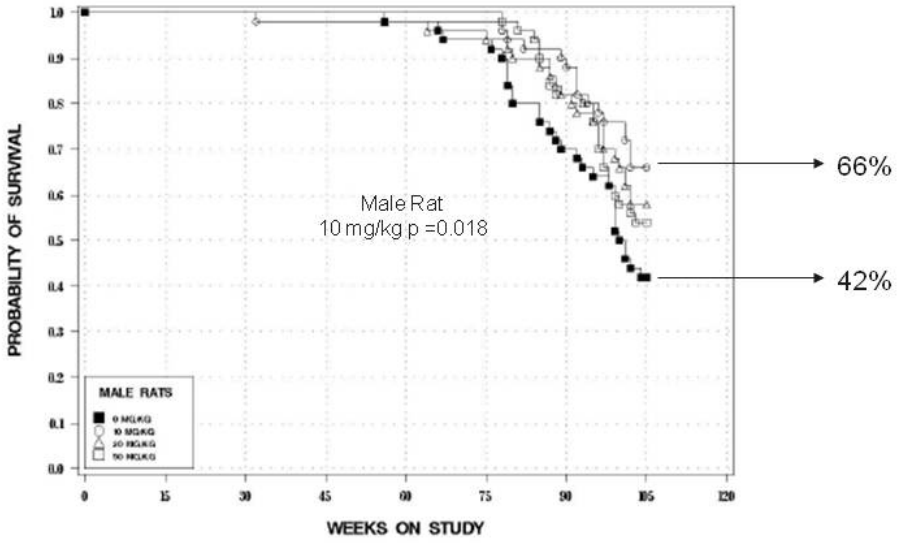
# F344/N Rats Survival Curves during Chronic Exposure




**No treatment effects on survival in female rats (76, 74, 66, 74%)**

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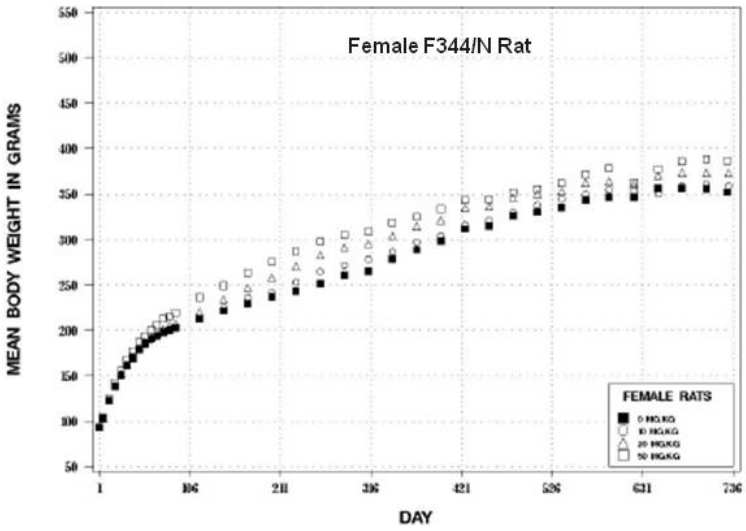
# F344/N Rats Growth Curve during Chronic Exposure




No treatment effects in male rat growth

# Mononuclear Cell Leukemia Incidence in F344/N Rats

| | *0 mg/kg* | *10 mg/kg* | *20 mg/kg* | *50 mg/kg* |
|---|---|---|---|---|
| **Male** | 26 | 22 | 18* | 18* |
| **Female** | 5* (10%) | 11(22%) | 18*** (36%) | 15*(30%) |

* $p < 0.05$, *** $p < 0.001$
Female HC: Same route 17% (10-24%), all routes 22% (8-40%); Historical Controls from same route and all routes includes the current control
* in control group indicates statistically significant trend

# Alveolar/bronchiolar Neoplasm Incidences in the F344/N Rat

| | 0 mg/kg | 10 mg/kg | 20 mg/kg | 50 mg/kg |
|---|---|---|---|---|
| Male | | | | |
| Adenomas | 0 | 0 | 5* (10%) | 2 (4%) |
| Adenoma or Carcinoma | 0 | 0 | 5* (10%) | 3 (6%) |

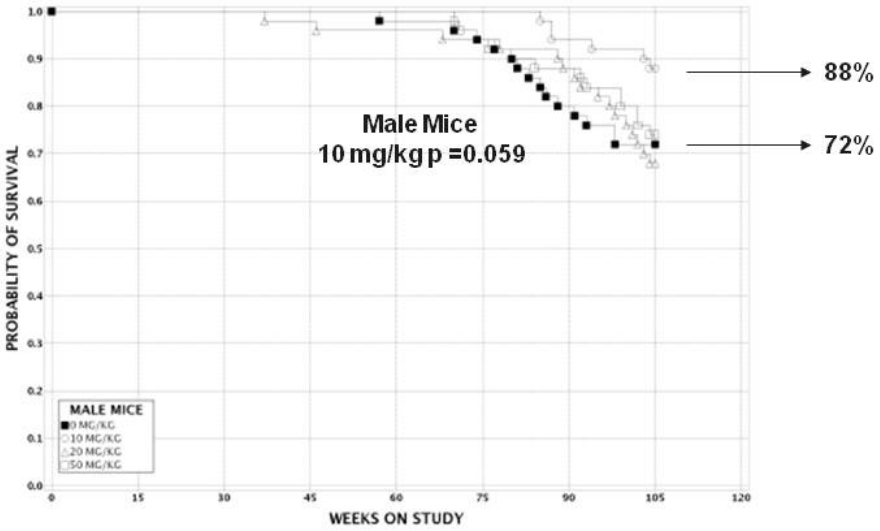
* $p < 0.05$
Adenomas: Same route 1.0% (0-2%), all routes 2.4% (0-8%)
Adenomas or Carcinomas: Same route 1.0% (0-2%), all routes 3.4% (0-10%)

# Decreasing Incidences of Lesions in the F344/N Rat

| | 0 mg/kg | 10 mg/kg | 20 mg/kg | 50 mg/kg |
|---|---|---|---|---|
| Male Testis | | | | |
| Testis Interstitial Cell Adenoma | 42*** | 39 | 36 | 26*** |
| Bilateral Adenoma | 29 | 29 | 28 | 10** |
| Female Mammary Gland | | | | |
| Multiple Fibroadenomas | 17 | 13 | 6** | 3** |
| Fibroadenoma | 35*** | 31 | 22** | 12*** |
| Fibroadenomas, Adenoma, or Carcinomas | 37*** | 32 | 22** | 12*** |
| Hyperplasia | 48 | 40** | 35** | 23** |
| Cyst | 15 | 3** | 9 | 3** |

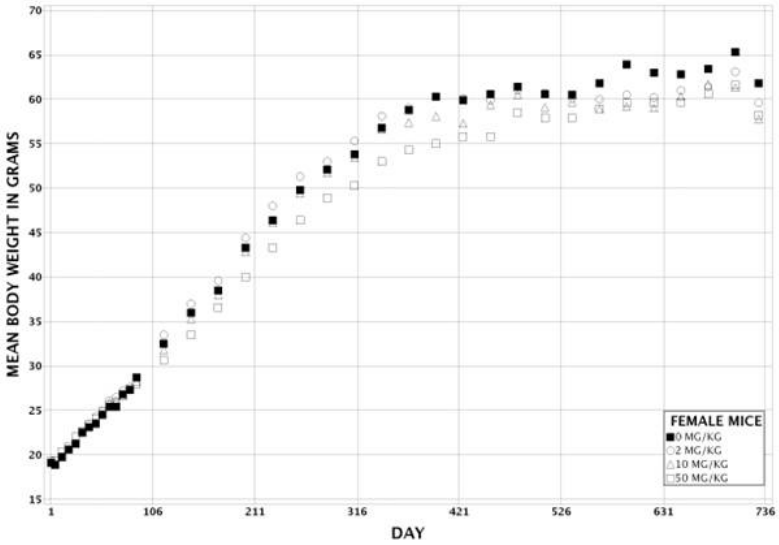
** $p < 0.01$, *** $p < 0.001$
* in control group indicates statistically significant trend

# B6C3F1 Mice Survival




No treatment effects on female survival (73, 80, 82, 82%)

# B6C3F1 Mice Growth




**No treatment effects on male mice growth**

**10 and 50 mg/kg/d Female mice weights were lower than controls**

# Hepatocellular Neoplasm Incidence in Male B6C3F1 Mice

| | 0 mg/kg | 10 mg/kg | 20 mg/kg | 50 mg/kg |
|---|---|---|---|---|
| **Multiple Adenoma** | 16 | 27* | 23 | 34** |
| **Adenomas** | 32** | 38 | 29 | 43** |
| **Multiple Carcinoma** | 7 | 12** | 10 | 17** |
| **Adenoma or Carcinoma** | 41* | 47 | 42 | 48* |
| **Multiple Hepatoblastoma** | 0 | 1 | 1 | 3 |
| **Hepatoblastoma** | 3 (6%) | 8 (16%) | 7 (14%) | 8 (16%) |
| **Adenoma, Carcinoma, Hepatoblastoma** | 41* | 47 | 43 | 48* |

*p < 0.05, ** p < 0.01
Hepatoblastomas: Same route 5.0% (4-6%), all routes 3.3% (0-34%)
* in control group indicates statistically significant trend

# Hepatocellular Neoplasm incidence in Female B6C3F1 Mice

| | *0 mg/kg* | *2 mg/kg* | *10 mg/kg* | *50 mg/kg* |
|---|---|---|---|---|
| **Multiple Adenoma** | 4 | 7 | 7 | 17** |
| **Adenomas** | 14*** | 16 | 18 | 28** |
| **Multiple Carcinoma** | 1 | 2 | 5 | 4 |
| **Carcinoma** | 5 | 13* | 15* | 15* |
| **Adenoma or Carcinoma** | 17** | 23 | 27 | 32*** |

* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$
* in control group indicates statistically significant trend

# Pancreatic Lesions in B6C3F1 Mice

| *Islet Cell Adenoma* | *0 mg/kg* | *10 mg/kg* | *20 mg/kg* | *50 mg/kg* |
|---|---|---|---|---|
| **Male** | 2 (4%) | 2 (4%) | 2 (4%) | 5 (10%) |
| **Multiple Adenoma** | 0 | 0 | 0 | 1 |
| **Day of 1st incidence** | 729 (T) | 729 (T) | 620 | 493 |
| | **0 mg/kg** | **2 mg/kg** | **10 mg/kg** | **50 mg/kg** |
| **Female** | 0 | 2 (4%) | 4 (8%) | 4 (8%) |

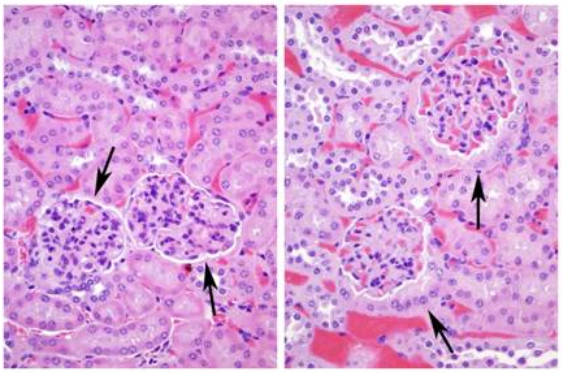
Male: Same route 2.0% (0-4%), all routes 1.2% (0-6%)
Female: Same route 1.1% (0-2%), all routes 0.8% (0-2%)

# Masculinization in Female B6C3F1 mice

| | 0 mg/kg | 2 mg/kg | 10 mg/kg | 50 mg/kg |
|---|---|---|---|---|
| Glomerulus Metaplasia | 2 (1.5) | 1 (2.0) | 5 (1.0) | 27** (2.0) |
| Submandibular Salivary Gland Cytopl. Alter. | 0 | 17** (1.2) | 40** (1.4) | 45** (2.5) |

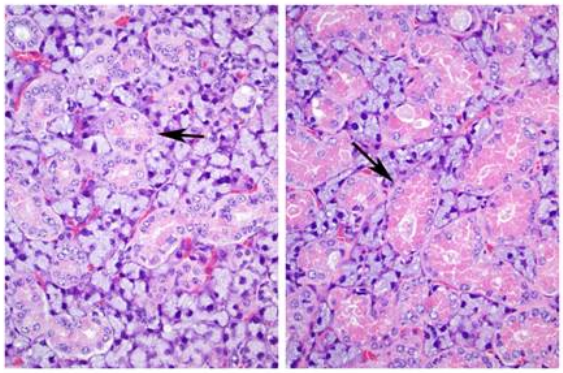
** $p < 0.01$

1=minimal, 2=mild, 3=moderate, 4=marked



Control Female 50 mg/kg/d Female

**Glomerulus Metaplasia**



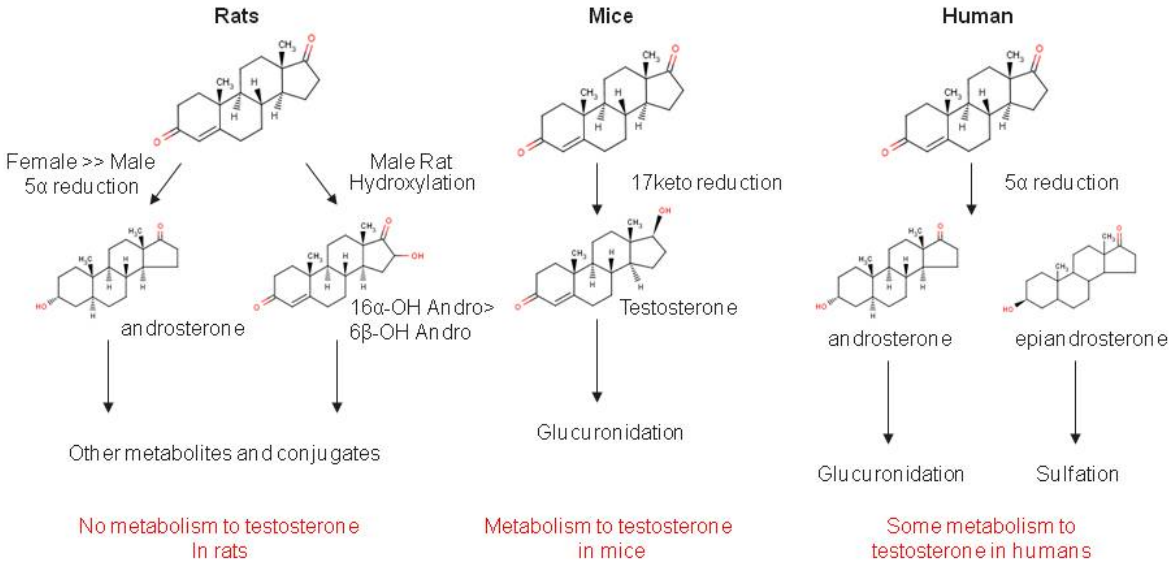
Control Female 50 mg/kg/d Female

**Submandibular Salivary Gland Cytoplasmic Alteration**

# Chronic Study Conclusions

- Equivocal evidence of carcinogenic activity in male F344/N rats:
  - Increase incidence of alveolar/bronchiolar adenomas and combined adenomas and carcinomas
- Equivocal evidence of carcinogenic activity in female F344/N rats:
  - Increased incidence of mononuclear cell leukemia
- Clear evidence of carcinogenic activity in male and female B6C3F1 mice:
  - Increased incidence of hepatocellular neoplasm multiplicity and total combined neoplasms (males). Increased incidence of hepatocellular adenomas and carcinomas (females)
  - Increased incidence of pancreatic islet cell adenomas were also considered related to treatment

# Androstenedione Metabolism in Hepatocytes (major pathways)




5α reduction intermediate 5α-androstanedione not shown